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Antiretroviral Exposure and Lymphocyte mtDNA Content Among Uninfected Infants of HIV-1-Infected Women

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Abstract

OBJECTIVE—Concern for potential adverse effects of antiretroviral (ARV) chemotherapy used to prevent mother-to-child HIV transmission has led the US Public Health Service to recommend long-term follow-up of ARV-exposed children. Nucleoside reverse transcriptase inhibitor ARV agents can inhibit DNA polymerase γ , impairing mitochondrial DNA (mtDNA) synthesis and resulting in depletion or dysfunction.

METHODS—We measured the mtDNA content of stored peripheral blood mononuclear cells (PBMCs) of 411 healthy children who were born to HIV-uninfected women and 213 uninfected infants who were born to HIV-infected women with or without in utero and neonatal ARV exposure. Cryopreserved PBMC mtDNA was quantified by using the Primagen Retina Mitox assay.

RESULTS—Geometric mean PBMC mtDNA levels were lower at birth in infants who were born to HIV-infected women. Among HIV-exposed children, mtDNA levels were lowest in those who were not exposed to ARVs, higher in those with exposure to zidovudine alone, and higher still in those with combination nucleoside reverse transcriptase inhibitor exposure. A similar pattern was observed in the corresponding women. Levels of mtDNA increased during the first 5 years of life in all HIV-exposed children but achieved normal levels only in those with ARV exposure.

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CONCLUSIONS—Levels of mtDNA are lower than normal in HIV-exposed children. Contrary to expectation, PBMC mtDNA levels are significantly higher in ARV-exposed, HIV-uninfected infants and their infected mothers compared with ARV-unexposed infants and women. By 5 years, levels of PBMC mtDNA rise to normal concentrations in ARV-exposed children but remain depressed in ARV-unexposed children.

Keywords

HIV; mitochondria; antiretroviral agents

All recommended antiretroviral (ARV) regimens to prevent mother-to-child transmission include zidovudine (ZDV), a nucleoside reverse transcriptase inhibitor (NRTI). NRTIs readily cross the placental and inhibit DNA polymerase γ , potentially interfering with fetal mitochondrial DNA (mtDNA) synthesis, resulting in mitochondrial depletion and/or dysfunction.¹ ARV-associated decreases in mtDNA have been reported, but correlations with mitochondrial disease have been inconsistent.^{2–9}

ARV exposure in utero has been associated with short-term and long-term mitochondrial dysfunction^{10–13} and with lower levels of blood mtDNA.^{14–16} Although large epidemiologic studies have found the incidence of clinically obvious mitochondrial disease rare,^{17–20} such reports are remarkably consistent with effects observed in vitro and in fetal monkeys.^{21–23} Studies of HIV-uninfected populations indicated that abnormal fetal growth is associated with lower peripheral blood mononuclear cell (PBMC) mtDNA levels,²⁴ suggesting that these levels may be a marker of in utero stress. Thus, concerns about in utero ARV exposure and mtDNA persist.

We measured PBMC mtDNA levels in HIV-positive mothers, in their HIV-uninfected infants, and in a comparison group of children who were born to healthy women. Our investigation was designed to evaluate the effects on infant PBMC mtDNA levels of maternal HIV infection and fetal and neonatal ARV exposure.

METHODS

Study Population

Samples from HIV-positive women and their uninfected infants were selected from participants in the National Institutes of Health Women and Infants Transmission study (WITS).²⁵ Infection status was determined according to the WITS protocol.²⁶ Samples were selected to reflect differential use of ARVs in pregnancy: no ARV exposure, exposure to ZDV alone, and exposure to combination ZDV and lamivudine with or without additional antiretrovirals (combination ARV [cARV]). From among subject pairs who met the selection criteria and had sufficient stored material available, efforts were made to balance within each time period the numbers with ARV exposure during the third trimester only, during the second and third trimesters only, or during all 3. No matching criteria were applied. The sample size of ~70 per group was established to provide 90% power to detect a 40% reduction in infant PBMC mtDNA in a 3-group comparison. Samples from healthy children who were born to HIV-uninfected women were obtained from an observational, cross-sectional study (P1009) of lymphocyte subsets in children aged 0 to 18 years conducted at sites that included all of the WITS centers.²⁷

Sample Collection and Processing

Blood from subjects in the WITS was collected with heparin anticoagulant before 1998, after which acid-citrate-dextrose solution B (ACD-B) was used. In P1009, EDTA anticoagulant was used. Both studies used identical protocols for processing, freezing, and storage of PBMCs.²⁸

Quantification of mtDNA

mtDNA was measured by a single reference laboratory (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA) that was blind to subject exposure status, using the Primagen MITOX assay (Amsterdam, Netherlands) according to the manufacturer's instructions. PBMC specimens were washed twice and pelleted before nucleic acid extraction. This method removes contaminating platelets.²⁹ Blinded duplicate samples were analyzed at Primagen to confirm results and ensure lack of platelet contamination.

MITOX measures cellular mtDNA levels by using a real-time duplex nucleic acid sequence-based amplification assay, in which mtDNA and nuclear DNA (nDNA) are amplified simultaneously in a single tube to maximize assay precision. Cellular mtDNA content is calculated as the ratio of mtDNA over nDNA and expressed as mtDNA copies per cell.²⁹

Statistical Analysis

Statistical analyses were performed by using SAS (Cary, NC). The χ^2 statistic was used for comparisons of proportions. Means were compared using the F test or *t* test as appropriate. For all statistical analyses, mtDNA values were logarithmically transformed. All results are reported as geometric mean values. Longitudinal data comparing age trends of mtDNA levels were analyzed by using the Proc Mixed procedure.^{30,31} Statistical comparisons assume a 2-tailed test with .05 α .

RESULTS

Characteristics of Cohorts

Stored PBMCs were available on 213 mother-infant pairs with different ARV exposures: 71 were ARV-naive, 71 received ZDV alone, and 71 received cARV. No infant whose mother was ARV-naive received ARV. All infants whose mother received ARV also received ZDV for the first 6 weeks of life. No ARV-exposed child had clinical mitochondrial disease. Specific maternal ARV use reflected temporal changes in recommendations of prevention of mother-to-child transmission.³² More than 90% ($n = 65$) of the HIV-positive women who received no ARV enrolled before March 1994. Women who received ZDV alone enrolled after March 1, 1994. All women but 1 who received cARV enrolled after August 1, 1996.

A demographic and clinical comparison among the 3 groups is presented in Table 1. No significant differences are present with respect to maternal age, race/ethnicity, Centers for Disease Control and Prevention HIV disease classification, or smoking. ARV-naive women had significantly higher CD4⁺ T-cell counts before delivery compared with women who were taking ZDV or cARV (626 vs 486 vs 448; $P = .018$), but the proportion with absolute CD4⁺ T-cell counts <200/ μ L did not differ significantly. Plasma HIV RNA levels before and at delivery were lower in women who were taking cARV.

Use of "hard drugs" (cocaine, crack, heroin, methadone, or injection drug use) was significantly different among the 3 groups ($P < .001$) and was highest in the ARV-naive group, consistent with temporal trends in drug use. Likewise, alcohol use was greatest in the no ARV group and least in the cARV group, but the difference was not statistically significant. Despite differences in hard drug use, frequencies of preterm (<37 weeks' gestation) birth were not significantly different among groups, and infant birth weight tended to be greater in the ARV-naive group ($P = .052$).

Prenatal cARV Exposures

ZDV and lamivudine were the only ARVs received by 30 of 71 women in the cARV group. Nineteen women in this group received other dideoxynucleosides in addition to ZDV/

lamivudine: 10 received stavudine, 4 received zalcitabine, 3 received didanosine, and 2 received both didanosine and stavudine (Table 2). The additional dideoxynucleoside exposure occurred during all 3 trimesters in 16 of 19 subjects. Nearly one half (33 of 71) of the women received a protease inhibitor, most frequently nelfinavir (22 of 33). Approximately 40% (13 of 33) of women who were treated with protease inhibitors received them through all trimesters of pregnancy.

Levels of mtDNA in Healthy Children

Levels of PBMC mtDNA were determined in 411 healthy, HIV-unexposed children who were aged birth to 18 years and participated in P1009.²⁷ Approximately half (48.9%) of the subjects were female; 56% were black, 33% were Hispanic, and 8% were white non-Hispanic. There were no age-related differences in PBMC mtDNA levels ($P = .87$; Fig 1). The overall distribution of their mtDNA values was used as the healthy control reference.

Levels of mtDNA at Birth

Figure 2 depicts levels of mtDNA in the PBMCs among 4 groups of HIV-uninfected children: (1) healthy children who were born to HIV-uninfected women (controls), (2) infants who were born to HIV-positive women and did not receive ARVs (no ARV), (3) infants who were born to HIV-positive women and were exposed in utero and postpartum to ZDV alone (ZDV), or (4) infants were exposed in utero to cARV (ZDV/lamivudine). Children who were exposed neither to HIV nor ARVs had the highest levels of mtDNA (528 copies per cell) compared with all other groups ($P < .001$). Among infants whose mother had HIV infection, levels of mtDNA at birth were higher in those who were exposed to ARVs (cARV: 321 copies per cell [$P < .001$]; ZDV alone: 268 copies per cell [$P = .01$]) compared with those who were not exposed to ARVs (197 copies per cell).

Effect of Duration of In Utero ARV Exposure on mtDNA Levels at Birth

We compared infant PBMC mtDNA levels at birth according to the duration of gestational ARV exposure. Exposure to cARV was associated with significantly higher mtDNA levels in all in utero exposure duration subcategories than children without ARV exposure (Table 3). Children who were exposed to ZDV alone, regardless of duration, had levels closer to ARV-unexposed children. Duration of ARV exposure was not associated with significantly different levels of mtDNA within any exposure category.

Levels of mtDNA at 2 and 5 Years of Age

To determine whether effects on infant mtDNA persisted, we analyzed mtDNA levels in HIV-exposed children from PBMC specimens collected at 2 and 5 years of age (Fig 2). Samples were available on all children at 2 years of age and on 53 children at 5 years of age. In contrast to control subjects, mtDNA levels in HIV-exposed children increased with age regardless of the specific ARV exposure. Children who were exposed to cARV had the highest levels, whereas those who were not exposed to ARV had the lowest, at all time points (birth: $P < .001$; 2 years: $P = .002$; 5 years: $P < .001$). Children who were exposed to ZDV alone had intermediate values at all time points. By 5 years, ARV-exposed children had levels comparable to those of control subjects. The rate of increase in PBMC mtDNA with age differed by exposure group. During the first 5 years, the annual increase in mtDNA was ~30 copies per cell in ARV-unexposed children and 60 copies per cell ARV-exposed children.

Maternal PBMC mtDNA Levels

Levels of PBMC mtDNA in HIV-positive women varied according to ARV exposure in a manner similar to their infants: ARV-naïve women had the lowest levels of mtDNA (218 copies per cell), and those who were taking cARV had the highest (317 copies per cell; $P = .0007$).

Women who were taking ZDV alone had intermediate levels (278 copies per cell), which were significantly greater than in the ARV-naïve group ($P = .027$) but not significantly different from women who were taking cARV ($P = .23$). Maternal and infant mtDNA levels at birth were significantly but loosely correlated in all groups, with only 19% of the variability in infant mtDNA levels explained by maternal mtDNA level ($P < .0001$, $R^2 = 0.19$).

Other Maternal and Infant Characteristics Associated with Infant mtDNA Levels

We used a mixed-model analysis of variance to examine the effects of the following maternal and infant variables on infant mtDNA: ARV exposure, maternal alcohol use, maternal cocaine/crack use, maternal hard drug use, maternal predelivery CD4 count, maternal predelivery plasma HIV RNA, maternal delivery CD4 count, maternal delivery CD4%, maternal delivery HIV RNA level, infant birth weight, and infant age. Only ARV exposure (ZDV alone: $P = .0011$; ZDV/lamivudine cART: $P = .0001$), infant age ($\beta = .2260$; $P = .0001$ at 5 years), and maternal CD4 count before delivery ($\beta = .0002$; $P = .0002$) were significantly associated with infant mtDNA levels. Maternal illicit drug use was not associated with infant mtDNA levels.

Effect of Freeze-Thaw and Anticoagulant

Specimens that were used in this study were collected by using various anticoagulants and stored for various lengths of time. Longer storage could be associated with DNA degradation, particularly if samples inadvertently were subjected to cycles of freezing and thawing.

To determine whether PBMCs that were collected with heparin or ACD-B differed in measured mtDNA content at baseline or after 2 freeze-thaw cycles, we compared in a blinded manner 45 samples from 13 healthy, HIV-uninfected volunteers. Although there was no significant difference in mtDNA content between paired heparin- and ACD-B–collected specimens at baseline, there was a nonsignificant trend for samples that were collected with heparin to have higher mtDNA levels after 2 freeze-thaw cycles.

DISCUSSION

We quantified PBMC mtDNA levels in 213 HIV-uninfected infants who were born to HIV-positive women (the study group) and 411 healthy children who were born to uninfected women (the control group). Children with in utero and neonatal exposure to ARVs had lower levels of mtDNA at birth compared with healthy children. These data confirm and extend earlier, smaller studies of humans and animals.^{14,15,22,33} In contrast to previous reports, our study included large numbers of children who were born to HIV-positive women who did not receive ARVs during pregnancy, and levels of mtDNA in these children exhibited even greater reductions. Contrary to our original hypothesis, levels of mtDNA in children who were born to untreated HIV-positive women were significantly lower than those who were exposed to ARVs. Moreover, children who were exposed to ZDV alone had lower levels of mtDNA compared with those who were exposed to cARV, suggesting that more potent ARV exposures have greater salutary effects on infant mtDNA levels.

HIV infection itself has been associated with decreased mtDNA levels and mitochondrial dysfunction in many tissue types, including PBMCs.^{8,34–36} The mechanisms by which this occurs are undefined but may involve altered mitochondrial membrane permeability by HIV proteins,³⁷ oxidative stress,² and/or a proinflammatory milieu.³⁸ Studies indicate that short-term ARV exposure in children and adults is associated with increases in mtDNA, whereas prolonged exposure—particularly to dideoxynucleosides (zalcitabine, stavudine, and didanosine)—results in marked decreases in mtDNA.^{2,4,29,39,40} This suggests that ARVs initially counteract the deleterious effects of HIV infection on mtDNA. The differences in maternal mtDNA that were observed in our study are consistent with this model; however,

none of the children in our study was HIV-positive, and this mechanism cannot be invoked to explain the salutary effects of ARVs on their mtDNA.

Although maternal and infant mtDNA levels in our study were significantly associated, the correlation was poor, indicating that infant mtDNA levels may be related to other factors and not simply reflective of maternal levels. In HIV-uninfected subjects, there was no correlation between infant and maternal PBMC mtDNA content, but infants with abnormal birth weight (small or large for gestational age) had lower cord PBMC mtDNA compared with infants who were born with appropriate weight for gestational age.²⁴ In our study, there was no difference among groups in the number of infants who were small or large for gestational age; however, adverse intrauterine environments have long been associated with programming effects on tissue morphology and function.⁴¹ The HIV-positive ARV-naïve women in our study had high CD4-positive T-cell counts (> 600 cells per μL), and the birth weight of their children was slightly greater than that of the ARV-exposed children. This suggests that the observed effect of ARVs on mtDNA is not associated with gross differences in fetal nutrition and may reflect other intrauterine disturbances. Protein malnutrition during fetal life has been associated with decreased mtDNA content⁴² and is believed to be a consequence of mitochondrial damage as a result of oxidative stress.^{43,44} Oxidative stress also has been suggested as a mechanism of HIV-induced mtDNA depletion, which is reversed with ARVs. This may be a mechanism by which maternal health affects infant mtDNA.

When stratified by duration of intrauterine exposure, birth mtDNA levels within each intrauterine exposure duration subcategory in the cARV group (but not the ZDV alone group) were significantly higher compared with HIV-exposed infants without ARV exposure. Among infants who were exposed to cARV, mtDNA levels were higher (but not statistically significantly different) in those with shorter exposures; however, most infants (19 of 25) who were exposed to ARVs during all 3 trimesters were exposed to additional dideoxynucleosides, whereas only 3 of 46 infants with shorter exposures were on these agents. Although not possible to dissociate the effects of duration of exposure and individual drug effects, it is striking that mtDNA levels were significantly higher in infants with relatively prolonged exposure to dideoxynucleoside drugs. This suggests that unchecked maternal HIV replication is more deleterious to infant mtDNA than prolonged fetal ARV exposure.

Levels of PBMC mtDNA increased in HIV-exposed children at a slow, seemingly constant rate during the first few years of life. By 5 years, mtDNA levels were normal in ARV-exposed children but remained significantly reduced in the ARV-unexposed children. In contrast, mtDNA levels in our control group did not vary with age, suggesting that this increase in mtDNA levels among HIV-exposed children represented recovery from intrauterine or perinatal insult. The rate of increase of 60 copies per cell per year in our subjects is within the range reported in adults and children who started on ARVs.^{2,40}

The strengths and limitations of this study merit consideration. It is the largest longitudinal study of mtDNA in HIV-exposed, uninfected children with variable ARV exposure. Given myriad effects of HIV on maternal health, the inclusion of an ARV-naïve group is an additional major strength. Another strength is the reference control group of healthy children who were born to HIV-uninfected women. In addition, all mtDNA levels were analyzed by a blinded single laboratory by using a validated assay that minimized platelet contamination, thereby ensuring accurate and nonbiased assessment.

Several limitations exist, however. Given the observational nature of our study, we cannot determine a causal relationship between ARVs and mtDNA levels. Like all observational studies, our study may be confounded by secular trends and lack of randomization. We did consider and adjust for a large number of potential confounders in analysis. Nonetheless, that

possible residual confounding affected our results cannot be excluded. In addition, specimens from untreated women were stored in liquid nitrogen vapor longer than those from subjects who were exposed to ARVs. Although our studies did not detect significant differences in mtDNA with repeated freeze-thaw cycles, it is theoretically possible that prolonged storage is associated with effects not related to temperature variation. As with most new diagnostic laboratory technologies, experimental data on analyte stability in long-term storage are lacking. A loss on the order of 0.03 log₁₀ mtDNA copies per cell per year of storage could negate our findings; however, nDNA is believed to be more vulnerable to degradation compared with mtDNA as a result of differences in size, location, and conformation.⁴⁵ Because our measurement of mtDNA was obtained by using the ratio of mtDNA to nDNA, time-dependent degradation, if it existed, theoretically would lower nDNA levels more than mtDNA levels, thereby increasing rather than decreasing the observed number of mtDNA copies per cell.

Only 11% of the children in the WITS were exposed to protease inhibitors, all received prenatal care, and their mother's CD4 counts were high, potentially limiting generalizability of our findings to populations that are treated with those regimens.

Finally, the clinical significance of decreased mtDNA levels in PBMCs is uncertain. ARV-induced depletion of PBMC mtDNA levels tends to be associated with clinical manifestations of mitochondrial toxicity but is not per se an adequate proxy for symptomatology or mtDNA content of other tissues.^{2-9,46,47} None of the children in this study had clinical symptoms consistent with mitochondrial dysfunction, despite markedly lower levels of mtDNA after many years; however, the long-term consequences of decreased mtDNA levels are unknown. In HIV-uninfected populations, decreased PBMC mtDNA has been implicated in the development of type 2 diabetes, and with metabolic syndrome in adulthood.^{42,48}

Despite these caveats, our data suggest that uncontrolled maternal HIV infection and ARV exposure have significant opposing, prolonged effects on the developing fetus. The net result of these effects on infant mtDNA levels depends on both duration and type of ARV exposure, but, overall, short-term ARV exposure seems to be beneficial.

CONCLUSIONS

Our data highlight the potential fetal adverse effects of uncontrolled maternal HIV replication and the need for continued surveillance and other investigations to monitor effects of in utero HIV and ARV exposure.

WHAT'S KNOWN ON THIS SUBJECT

Results of in vitro and animal studies have suggested that in utero exposure to ARVs decreases mitochondria. Decreases in mtDNA have been reported in ARV-exposed infants, but correlations with mitochondrial disease have been inconsistent.

WHAT THIS STUDY ADDS

In contrast to other studies, we included children who were born to HIV-infected women who did not receive ARV as well as those who received ZDV alone or in combination with other drugs, and we quantified mtDNA in normal healthy children.

ABBREVIATIONS

ARV	antiretroviral
ZDV	zidovudine

NRTI	nucleoside reverse transcriptase inhibitor
mtDNA	mitochondrial DNA
PBMC	peripheral blood mononuclear cell
WITS	Women and Infants Transmission Study
cARV	combination antiretroviral
ACD-B	acid-citrate-dextrose solution B
nDNA	nuclear DNA

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FIGURE 1.

Levels of mtDNA in the PBMCs of 411 healthy children who were born to HIV-uninfected women. Shown is a scatterplot of PBMC mtDNA levels on a logarithmic scale in healthy children, according to age. Each circle represents a single individual; the solid line denotes the mean.

FIGURE 2.

Box-and-whisker plots on a logarithmic scale of PBMC mtDNA levels in healthy children and in HIV-exposed, uninfected children, according to exposure category and age. Upper and lower box borders represent the 75th and 25th percentiles, solid lines represent the median, dashed lines represent the mean, and error bars represent the 10th and 90th percentiles. ^a $P < .001$ versus controls; ^b $P < .02$ versus no ARV exposure group within same age category; ^c $P < .001$ versus no ARV exposure group within same age category. 3TC indicates lamivudine.

TABLE 1
Demographic and Clinical Characteristics of WITS Maternal and Infant Study Subjects According to ARV Exposure Category

Characteristic	No ARV Therapy			ZDV Monotherapy			ZDV/Lamivudine cARV Therapy			p ^a
	N	n	% or Mean	N	n	% or Mean	N	n	% or Mean	
Age at delivery, y	71		28.02	71		26.08	71		29.18	.252
Race	71			71			71			.789
White	6		8.45	7		9.85	8		11.26	
Black	39		54.92	30		42.25	30		42.25	
Hispanic	23		32.39	30		42.25	29		40.84	
Other	3		4.22	4		5.63	4		5.63	
CD4 count at delivery, per μ L	69		625.92	63		479.47	64		530.65	.083
CD4 <200/ μ L at delivery	69	7	10.14	63	9	14.28	64	8	12.50	.766
CD4% at delivery	69		31.56	63		28.52	66		29.65	.319
CD4 count before delivery, per μ L	51		626.64	54		486.44	56		448.58	.018
CD4 <200/ μ L before delivery	51	4	7.84	54	8	14.81	56	5	8.92	.451
CD4% before delivery	51		32.43	54		28.57	56		27.73	.027
HIV RNA at delivery, log ₁₀ copies per mL	66		3.70	67		3.69	64		3.11	<.001
RNA before delivery, log ₁₀ copies per mL	51		3.54	53		3.67	56		3.15	.006
Hard drug use	71	34	47.88	71	22	30.98	71	13	18.3	<.001
Injection drug use	66	5	7.57	69	2	2.89	68	1	1.47	.165
Cocaine/crack use	71	28	39.43	70	15	21.42	68	6	8.82	<.001
Alcohol use	71	34	47.88	71	27	38.02	71	20	28.16	.053
Smoking	71	37	52.11	71	29	40.84	71	25	35.21	.116
CDC HIV disease stage classification	71			71			71			.518
Class C	3		4.22	8		11.26	4		5.63	
Class B	16		22.53	13		18.30	16		22.53	
None	52		73.23	50		70.42	51		71.83	
Infant birth weight, kg	67		3.19	69		3.01	70		3.10	.343
Infant birth weight < 2.5 kg	67	6	8.95	69	10	14.49	70	5	7.14	.330
Infant birth weight < 1.5 kg	67	0	0.00	69	0	0.00	70	0	0.00	

Characteristic	No ARV Therapy			ZDV Monotherapy			ZDV/Lamivudine cARV Therapy			<i>P^a</i>
	<i>N</i>	<i>n</i>	% or Mean	<i>N</i>	<i>n</i>	% or Mean	<i>N</i>	<i>n</i>	% or Mean	
Prematurity (< 37 wk gestation)	71	10	14.08	71	6	8.45	71	6	8.45	.444

“At delivery” is defined as delivery date ± 14 days. “Before delivery” is defined as 15 to 90 days before delivery date. “Hard drug use” is defined as any of the following: cocaine, crack, heroin, methadone, or injection drug use.

^a*P* value from a χ^2 or F test statistic comparing distributions among the 3 groups.

TABLE 2

Prenatal Combination ARV Regimens Among Women Who Received ZDV/Lamivudine

Parameter	Third Trimester Only	Second and Third Trimesters	All Trimesters	Total
ZDV + 3TC	5	18	7	30
ZDV + 3TC + d4T			1	2
ZDV + 3TC + ddC			2	2
ZDV + 3TC + ddI			1	1
ZDV + 3TC + IDV	1			1
ZDV + 3TC + NFV	4	10		14
ZDV + 3TC + NVP		1		1
ZDV + 3TC + RTV	1	1		2
ZDV + 3TC + SQV	1	1		2
ZDV + 3TC + d4T + IDV			1	1
ZDV + 3TC + d4T + NFV		1	1	2
ZDV + 3TC + d4T + NVP	1		1	2
ZDV + 3TC + ddI + IDV			1	1
ZDV + 3TC + ddC + NFV			1	1
ZDV + 3TC + ddC + SQV			1	1
ZDV + 3TC + IDV + SQV			1	1
ZDV + 3TC + NFV + SQV			1	1
ZDV + 3TC + d4T + IDV + SQV			1	1
ZDV + 3TC + d4T + NFV + NVP			1	1
ZDV + 3TC + d4T + NFV + EFV			1	1
ZDV + 3TC + ddI + NFV + NVP			1	1
ZDV + 3TC + d4T + ddI + IDV + NVP			1	1
ZDV + 3TC + d4T + ddI + NFV + NVP			1	1
Total	13	33	25	71

3TC indicates lamivudine; d4T, stavudine; ddC, zalcitabine; IDV, indinavir; ddI, didanosine; NFV, nelfinavir; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir; EFV, efavirenz.

TABLE 3
Infant PBMC mtDNA Levels at Birth According to ARV Exposure Category and Duration

Parameter	No ARV			ZDV Monotherapy			ZDV/Lamivudine cARV Therapy		
	<i>n</i>	Geometric Mean mtDNA ^a	95% CI	<i>n</i>	Geometric Mean mtDNA ^a	95% CI	<i>n</i>	Geometric Mean mtDNA ^a	95% CI
Third trimester only	0	NA		24	250.8	196.7–319.8	13	399.9	286.4–558.6
Second and third trimesters	0	NA		23	235.6	183.6–302.3	33	306.3	251.1–373.9
All trimesters	71	196.5	170.2–226.8	24	235.6	183.3–302.9	25	267.4	213.2–335.4
Total	71	196.5	170.2–226.8	71	240.7	208.3–278.2	71	306.6	267.2–351.7

P values represent comparison of the indicated cell with the “No ARV, Total” mtDNA level. Within a given exposure category, there were no statistically significant differences for any pairwise comparison of mtDNA according to duration of fetal exposure (*P* values not shown). CI indicates confidence interval; NA, not applicable.

^aCopies per cell.